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Research Paper

Aloe ferox, *Agathosma betulina*, and *Bulbine frutescens* Extracts Show Antifungal Activity at Concentrations Favourable to the Proliferation of Epidermal Cells

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ABSTRACT

Introduction: The increasing prevalence of antifungal resistance presents an enormous challenge to healthcare professionals. The adverse effects and resistance of current antifungal drugs have prompted the need for alternate antifungal therapy in the form of natural products. Although anecdotal reports have suggested that *Aloe ferox*, *Agathosma betulina,* and *Bulbine frutescens* have been used to successfully treat fungal skin infections, little scientific evidence is available to validate these claims. Therefore, this study aimed to determine whether the concentrations of *A ferox*, *A betulina,* and *B frutescens* hydroethanolic extracts showing antifungal activity against skin-related *Candida* species are cytotoxic to normal human epidermal cells.

Methods: Crystal violet and lactate dehydrogenase assays were employed to determine cell viability and cytotoxicity on the human epidermal cells. Broth microdilutions were performed to evaluate the susceptibility of *Candida* species to plant extracts.

Results: Candida spp. inhibition was observed at a concentration of 200 µg/ml, while optimum extract concentrations for cell proliferation ranged between 200 and 400 µg/ml for *B frutescens* and 200 µg/ml for *A ferox* and *A betulina*. This suggests that these plants could prove to be effective as antifungal agents at a dosage of 200 µg/ml while still supporting the growth of epidermal cells at the same concentration, which is an important process in maintaining healthy skin.

Conclusions: These findings from this study lay a foundation for further studies confirming the applicability of these medicinal plants as safe alternative antifungal treatments that promote skin cell proliferation, regeneration, and enhance tissue repair.

Introduction

The incidence of fungal infections is increasing at an alarming rate, presenting an enormous challenge to healthcare professionals. Superficial mycoses such as candidiasis are skin diseases with a great prevalence affecting between 20% and 25% of people globally (Gnat et al., 2021). Cutaneous candidiasis is a fungal infection of the skin, hair, and nails caused by *Candida* species, which are opportunistic pathogens occurring as predisposing factors in immunosuppressive diseases (Declara et al., 2022). More than 30 *Candida* species reportedly cause infections in humans, with *Candida albicans* being the most prevalent species of the skin microbiome (Kühbacher et al., 2017). Other non-albicans species such as *Candida tropicalis*, *Candida dubliniensis,* and *Candida glabrata* have also been isolated from the skin of persons with

Abbreviations: A betulina, Agathosma betulina; *A ferox*, Aloe ferox; *B frutescens*, Bulbine frutescens; *C albicans*, Candida albicans; *C dubliniensis*, Candida dubliniensis; *C glabrata*, Candida glabrata; *C tropicalis*, Candida tropicalis; CV, crystal violet; DMEM, Dulbecco's Modified Eagle's Medium; H&E, hematoxylin and eosin; HaCaT, human epidermal cells; IC50, inhibitory concentration IC50; LDH, lactate dehydrogenase; MIC, minimal inhibitory concentrations

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underlying immunosuppressive conditions such as HIV/AIDS and those undergoing chemotherapy (Espinosa-Hernández et al., 2020).

Fluconazole is one of the most commonly used drugs for the treatment of candidiasis. However, fluconazole only has fungistatic activity against species such as *C albicans* (Lu et al., 2021). The global increase in azole-resistant *Candida* strains poses a major challenge in treating fungal infections. High levels of primary resistance to fluconazole have been reported against *C albicans*, *C glabrata,* and *C tropicalis* (Jin et al., 2018; Lu et al., 2021).

Azole resistance may be attributed to fungal biofilm formation. Biofilms are complex surface-associated microbial cell populations embedded in an extracellular matrix that attaches to tissue surfaces as well as solid surfaces (e.g., catheters or prostheses) (Silva et al., 2012). *Candida* biofilms can adhere to the surfaces of medical devices (Bizerra et al., 2008; Silva et al., 2012). The eradication of fungal biofilms requires prolonged use of antimicrobial therapy that can result in drug tolerance. This is a major cause of biofilm-related antifungal resistance, as an estimated 65–80% of all fungal infections are biofilm-related (Macià et al., 2018). The ideal antifungal agents should have a broad spectrum of fungicidal activity, be selective to host cells, and specifically target biofilm-associated infections (Pereira, 2021; Wong et al., 2014).

Medicinal plants have been used for the treatment of skin conditions for many years, with ingredients from plant extracts forming the largest categories of cosmetic skincare formulae (Gediya et al., 2011; Sharma et al., 2003; Wilkinson and Brown, 1999). South Africa (**SA**) has a rich diversity of plant species with approximately 117 species traditionally used to treat various skin disorders, including acne, boils, ringworm, sores and wounds (Lall and Kishore, 2014). Van Wyk (2008) reported that at least 90 of these SA medicinal plants are being considered for further development and commercialisation in skincare products. South Africa plants such as the African oil palm (*Elaeis guineensis* Jacq), Baobab (*Adansonia digitata*), Marula (*Sclerocarya birrea*), Sesame (*Sesamum indicum*), Bitter Aloe (*Aloe ferox*), Rooibos tea (*Aspalathus linearis*), and Honeybush tea (*Cyclopia intermedia*) are used in moisturising cosmetic formulations to treat various skin ailments (Lall and Kishore, 2014). A previous review (Ndlovu et al., 2021a) identified that plant species such as *Aloe ferox* (*A ferox*), *Agathosma betulina* (*A betulina*), and *Bulbine frutescens* (*B frutescens*) are gaining popularity among communities in the Western Cape for the treatment of fungal skin infections, and have been chosen for further investigation in this study.

Aloe ferox, commonly known as 'bitter aloe,' is a medicinal plant indigenous to the Cape coastal region of SA, occurring in Swellendam in the west and extending to the southern parts of KwaZulu-Natal in the east (Chen et al., 2012; Glen and Hardy, 2000). The ethanolic extracts of *A ferox* have been reported to treat eczema, pimples, venereal sores and arthritis (Frum and Viljoen, 2006). The plant extracts have reportedly inhibited the growth of *C albicans* and *Neisseria gonorrhoea* (Kambiz and Afolayan, 2008). The Aloe gels contain compounds such as aloin, which showed strong antimicrobial activity against a wide range of gram-negative and gram-positive bacterial and fungal strains (Zapata et al., 2013). *B frutescens*, also known as the 'burn jelly plant,' is widespread throughout parts of the Northern, Western and Eastern Cape regions. *B frutescens* extract has been used to promote cutaneous wound healing and to treat rheumatism, sciatica, and itching (Frum and Viljoen, 2006; Pather et al., 2011; Pather and Kramer, 2012). The leaf extracts of the plant reportedly inhibit gram-positive bacterial growth such as *Bacillus subtilis*, *Micrococcus kristinae*, and *Staphylococcus aureus* (Coopoosamy, 2011). *A betulina*, commonly referred to locally as 'buchu,' is found in the Cape region, and its essential oils are reportedly effective in the treatment of *Trichophyton rubrum* and *Trichophyton mentagrophytes* strains, which commonly cause athlete's foot and ringworm (Fajinmi et al., 2019; Lis-Balchin et al., 2001). The hydodistilled essential oils of this plant reportedly have antimicrobial activity against *S aureus*, *Bacillus cereus*, *Klebsiella pneumoniae*, and *C albicans* with minimal inhibitory concentration (**MIC**) values ranging between 2 and 4 mg/ml (Moolla et al., 2007; Viljoen et al., 2006).

Although there is anecdotal evidence showing that the above-mentioned plants have beneficial dermatological capabilities, there is still a need to scientifically evaluate the susceptibility of *Candida* species and to determine the MIC of these extracts. Few studies have investigated the extracts' effect on cell viability, cellular growth rates, and cytotoxicity when exposed to epidermal cells and barrier function. Moreover, safety concerns exist, such as determining therapeutic dosages that can be used on human skin without causing any harm. Thus, the *in vitro* screening of the proliferation and cytotoxicity of the extracts needs to be performed on selected human cell lines before promoting their use in cosmetic formulations. These procedures can provide safety data for consumers who choose to use natural products (Ribeiro et al., 2015). The present study aimed to determine whether the concentrations of *A ferox*, *A betulina*, and *B frutescens* hydroethanolic extracts showing antifungal activity against skin-related *Candida* species, namely *C tropicalis*, *C albicans*, *C dubliniensis*, and *C glabrata* are cytotoxic to normal human epidermal (HaCaT) cells.

Materials and Methods

Plant Extracts

The commercially prepared ethanol-water extracts of *A betulina* (100 mg/ml), *A ferox* (500 mg/ml), and *B frutescens* (500 mg/ml) were obtained from Parceval Pharmaceuticals (SA). For the cell culture experiments, the hydroethanolic extracts were diluted in complete Dulbecco's Modified Eagle's Medium (Cat no. BE12-709F, Lonza, Cape Town, SA) and used at physiologically relevant ($\leq$0.5 mg/ml) working concentrations of 0.2-0.8 mg/ml (Chang et al., 2021). For the microdilution broth assays, the hydroethanolic extracts were diluted in Roswell Park Memorial Institute (RPMI)-1640 medium with L-glutamine (Cat no. RPMI-STA, Capricorn Scientific, Ebsdorfergrund, Germany) to generate 0.2-0.8 mg/ml working solutions of *A betulina*, *A ferox*, and *B frutescens* extracts. For each experiment, the extracts were stored at 4 °C. The chemical profiling and characterisation of *A betulina, A ferox,* and *B frutescens* have been extensively reported, and the main compounds found in each extract were limonene, aloesin, and knipholone, respectively (Avula et al., 2021; Bodede and Prinsloo, 2020; Masondo and Makunga, 2019; Zhao et al., 2016).

Cell Culture Assays

Cell Line

The HaCaT cell line was purchased from Cell Line Service (Eppelheim, Germany). The cells were seeded and propagated in complete Dulbecco's Modified Eagle's Medium, supplemented with 10% heat-inactivated foetal bovine serum (Cat no. 12389962, Hyclone, Little Chalfont, UK), penicillin (100 U/ml), and streptomycin (100 mg/ml) (GIBCO, NY, USA) and maintained in a humidified incubator with 5% CO_2 at 37 °C.

Determination of Cell Proliferation and Viability

The crystal violet (**CV**) assay was used to determine the effects of the ethanolic extracts on cell proliferation and viability, as previously described (Saotome et al., 1989). This assay was also used to determine the optimal concentrations used to treat the cells. The optimised concentrations included 200, 400, 600, and 800 µg/ml of the plant extract for 24, 48, and 72 hours, respectively. Cell proliferation was determined using the triarylmethane dye that binds to ribose molecules such as DNA in the nuclei. The HaCaT cells were seeded into 96-well tissue test plates (Greiner Bio-one, Kremsmunster, Austria) at a density of 1×10^4 cells/well and allowed to adhere overnight. The cells were treated with the various concentrations of the three plant extracts for 24, 48, and 72 hours. Cells without the extracts served as a negative control, while 1% Triton-X 100 was used as a positive control, and cells containing 1% ethanol were used as a vehicle control. At the end of each period, the

cell proliferation levels were assessed using the CV assay (stock 0.1%: PBS) with 0.2% Triton-X 100 as the solubilising agent and detected using a microplate reader (Glomax Multi Detection System, Promega, Wisconsin, USA) at 570 nm.

Determination of Cytotoxicity

The lactate dehydrogenase (**LDH**) assay was used to determine cellular cytotoxicity following the methods of Ndlovu et al. (2021b). Lactate dehydrogenase is a cellular cytosolic enzyme released into the cell culture medium upon damage to the plasma membrane (Kumar et al., 2018). Lactate dehydrogenase activity was measured using an LDH-cytotoxicity kit (Cat no. 11644793001, Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Briefly, the HaCaT cells were seeded in 96-well plates at a density of 1×10^4 cells/well. After 24 hours, various concentrations of the plant extracts were added, and the cells were incubated for 24, 48, and 72 hours. At the end of each time point, the LDH activity was measured, and the absorbance was read at 490 nm using a microplate reader (Glomax Multi Detection System, Promega, Madison, USA).

Cellular Morphological Changes

To evaluate the cellular morphological changes after the HaCaT cells were treated with the extracts, a hematoxylin and eosin (**H&E**) cell stain was conducted as previously described (Vorster et al., 2012). Cells were seeded on heat-sterilised coverslips in 6-well plates at a density of 2.5×10^5 cells per well and allowed to attach overnight. Cells were subsequently exposed to different concentrations of the three plant extracts and appropriate controls for 24, 48, and 72 hours, respectively, after which the coverslips were fixed with Bouin's fixative (30 minutes) and 70% ethanol (20 minutes). Coverslips were rinsed with water, stained with hematoxylin (20 min), rinsed with water and 70% ethanol, and stained with eosin (7 minutes), then dehydrated stepwise with ethanol (70%, 96%, 100%) and xylene, mounted on microscope slides with Dibutylphthalate Polystyrene Xylene mountant (Cat no. 44581, Sigma–Aldrich, St. Louis, USA) and dried overnight. Qualitative evaluation (400 × magnification) was conducted using a 360 4i Nikon (Tokyo, Japan) microscope. The ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used to analyse and process the images for better resolution.

Microdilution Assay

Fungal Strains and Preparation

C albicans (ATCC 90028), *C dubliniensis* (NCPF 3949a), *C glabrata* (ATCC 26512), and *C tropicalis* (ATCC 950) were used in this study. *Candida* species were inoculated onto Sabouraud dextrose agar (Cat. no. 84088, Sigma–Aldrich, USA) and incubated for 24 hours at 37 ℃ to allow sufficient time for growth. These species were then subcultured and maintained on Sabouraud dextrose agar for the duration of the experiments.

Broth Microdilution Method

The MIC values of the extracts against susceptible fungi were determined using a modification of the broth microdilution method used by Eloff (1998) in accordance with CLSI guidelines (Nascente et al., 2009). Briefly, the test organisms were standardised to 0.5 McFarland standard, from which a standardised fungal stock was prepared from a 1:100 dilution with sterile RPMI-1640 with L-glutamine (Cat no. RPMI-STA, Capricorn Scientific, Ebsdorfergrund, Germany) broth to give a final inoculum of approximately 10^6 CFU/ml. The extracts at a 200 µg/ml starting concentration were added to the first wells and serially diluted to yield different test concentrations (0.19–200 µg/ml). The diluted fungal culture (100 µl) was added to each well. The antifungal, fluconazole, (200 µg/ml) (Cat no. F8929, Sigma–Aldrich, Germany) was used only as a positive control, at a concentration above established MICs (Ng'uni et al., 2022) and a negative control was included to determine if the solvent (ethanol) exhibited any antimicrobial effects on the fungi. Each microtiter plate was sealed with a sterile adhesive film to prevent evaporative loss of the plant extracts and incubated at 37 °C for 24 h. The solution of p-iodonitrotetrazolium violet (02 mg/ml) (INT) (Cat no. 58030, Sigma–Aldrich, Germany) at 40 µl was added to each well and incubated at 37 °C for 30 minutes. Following the addition of INT, the development of a pinkish colour (visualised with the naked eye) indicated microbial growth (viable fungi can convert INT to red formazan by reduction). The wells that remained clear/yellowish indicated inhibition of fungal growth. The MIC was recorded as the lowest concentration of the extract that prevented the appearance of visible growth of the microorganism after 24 hours of incubation (Penduka et al., 2011). The assay was performed in triplicate on alternate days.

Data Analysis

Using the SPSS 29.0 software (SPSS, Chicago, IL, USA), the data were reported as mean and standard error of mean from triplicate measurements. The statistical significance of differences among groups was evaluated using one-way analysis of variance. Additionally, in the cell viability assay, the median inhibitory concentration IC50 values were calculated from linear regression plots using Microsoft Excel software (Microsoft Corporation, Redmond, WA, USA). Statistical significance was accepted at the level of $P < 0.05$.

Results

Effects of the Different Plant Extracts on Cell Viability and Proliferation

The hydroethanolic extracts from *A ferox* (Fig. 1A) showed a significant ($P < 0.05$) increase of 16% in HaCaT cell proliferation at the lower dosage of 200 µg/ml after 48 hours and a 20% decrease in proliferation at higher concentrations (600 and 800 µg/ml) after longer exposure (72 hours) to the extract, compared to the negative control. The hydroethanolic extracts from *B frutescens* (Fig. 1B) displayed a significant ($P < 0.05$) decrease of 40% in cell proliferation at higher concentrations (800 µg/ml) after longer exposure (72 hours). However, cells exposed to lower concentrations (200 and 400 µg/ml) displayed a significant increase. The HaCaT cells exposed to *B frutescens* at a 400 µg/ml concentration had a 17% significant ($P < 0.05$) increase in cell proliferation after 24 hours compared to the control (Fig. 1B). The hydroethanolic extracts from *A betulina* (Fig. 1C) exhibited a significant ($P < 0.05$) time- and dose-dependent reduction on HaCaT cells. After 72 hours, a significant 24% decrease in cell proliferation was noted in concentrations above 600 and 800 µg/ml when compared to the control. The results showed that the *A betulina* extracts exhibited no significant change in the cell proliferation at lower dosages (200–600 µg/ml) after 24 and 48 hours of treatment. A significant ($P < 0.05$) decrease in cell proliferation was observed in cells exposed to the positive control (1% Triton-X 100) at all exposure times. The ethanol had minimal effect on HaCaT cell viability, as demonstrated by the lack of a significant change in the vehicle control compared to the negative control.

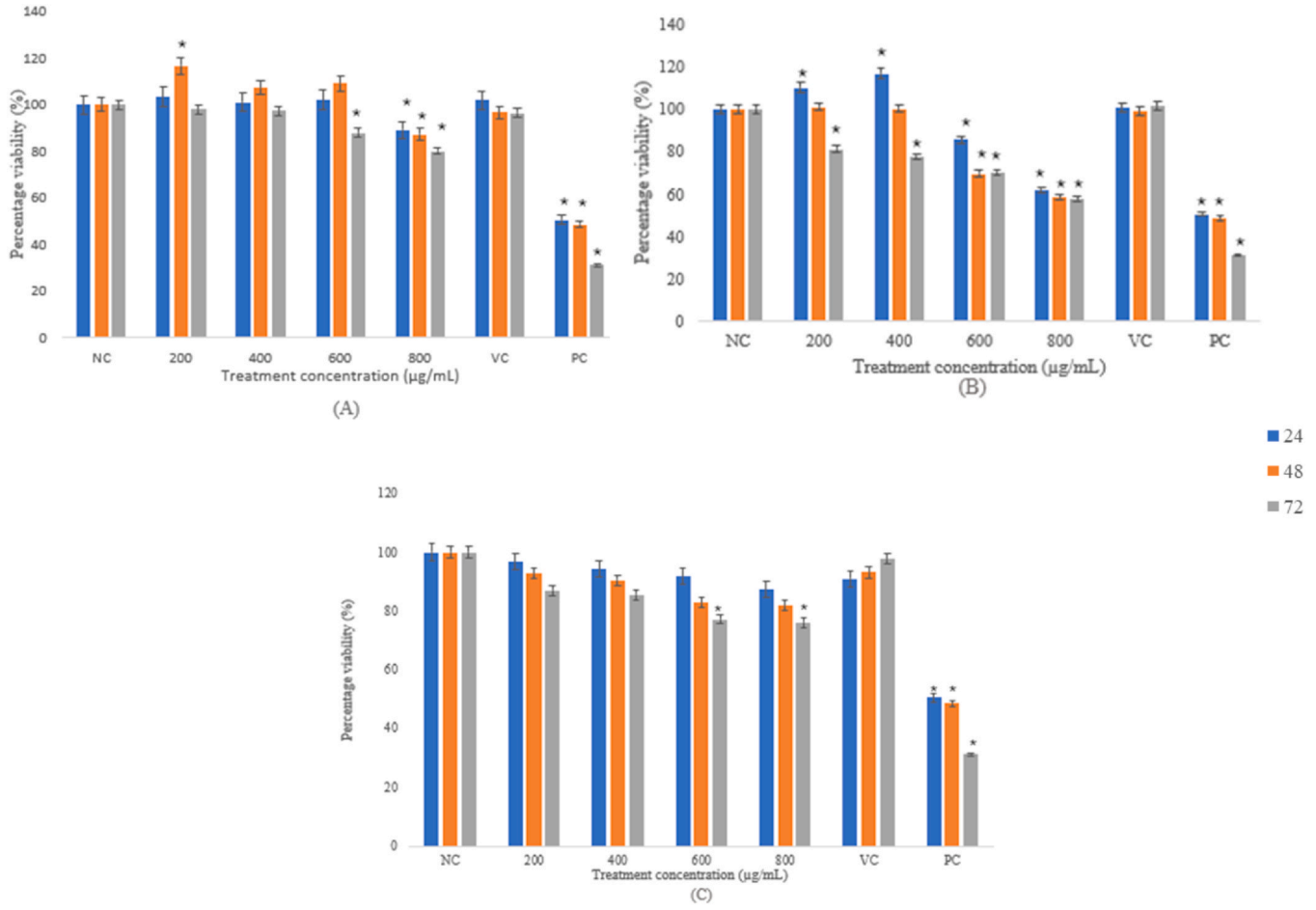

Fig. 1. Cell viability of normal skin (HaCaT) cells treated with 200, 400, 600 and 800 µg/ml concentrations of *Aloe ferox* (A), *Bulbine frutescens* (B) and *Agathosma betulina* (C) hydroethanolic extracts for 24, 48, and 72 hours determined using a crystal violet assay. NC, negative control—untreated cells with media; VC, vehicle control—cells treated with 1% ethanol; PC, positive control—cells treated with 1% Triton-X 100. Data represent the mean ± SEM, n = 3, where * indicates statistical significance ($P < 0.05$).

Potential Cytotoxic Effects of the Selected Plant Extracts

The results obtained from the LDH assay on the HaCaT cells are displayed in Fig. 2A-C and confirm that all three selected plant extracts exhibited negligible cytotoxicity towards the HaCaT cells after exposure to the various dosages at all time points compared to the negative control. The only significant ($P < 0.05$) increase in cytotoxicity was observed in the positive control at all exposure times.

Effects of the Plant Extracts on Cell Morphology

Hematoxylin and eosin staining revealed little or no morphological changes in the HaCaT cell line after exposure to various concentrations of the three extracts (*A ferox*, *A betulina*, and *B frutescens*) (Fig. 3). The results of the H&E staining further validated the data obtained from the CV and LDH assays in that the figures displayed mitotically active cells with intact nuclei and cytoplasmic properties across these concentrations and exposure times. Apoptotic characteristics (hypercondensed chromatin, membrane blebbing) were only observed in the positive control after 24, 48, and 72 hours compared to the negative control. The only significant ($P < 0.05$) increase in cytotoxicity was observed in the positive control at all exposure times.

Microdilution Assays

Table 1 annotates the MIC results showing that the hydroethanolic extracts had a broad spectrum of antifungal activity as they were able to inhibit the growth of the tested fungal strains between the ranges of 12.5–200 µg/ml. Fluconazole inhibited the growth of all the fungal strains tested, with MIC values of 200 µg/ml. The extracts of *A betulina* displayed antifungal activity against *C tropicalis* and *C albicans,* with MIC values of 12.5 and 50 µg/ml, respectively. *Aloe ferox* extracts

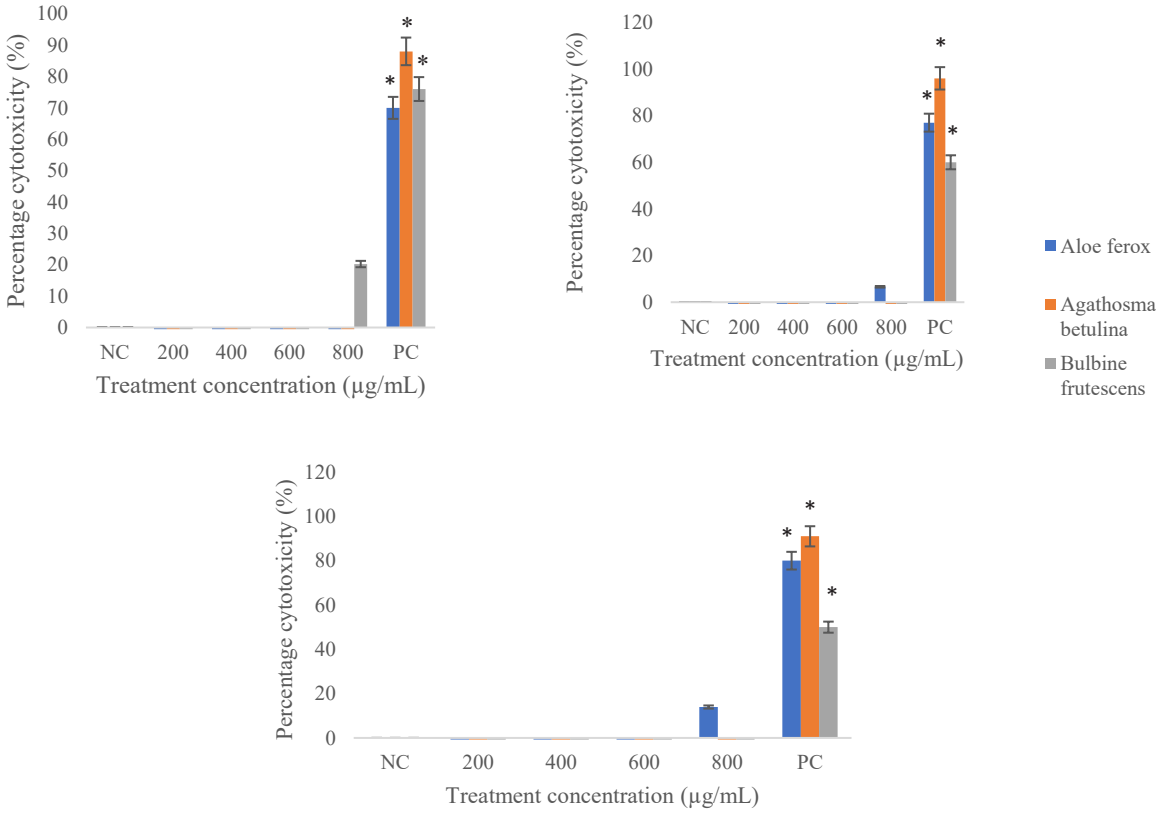

Fig. 2. Cell cytotoxicity was determined using the lactate dehydrogenase (LDH) assay. HaCaT cells were treated with 200, 400, 600, and 800 µg/ml concentrations of *Aloe ferox*, *Bulbine frutescens*, and *Agathosma betulina* hydroethanolic extracts for 24 (A), 48 (B), and 72 hours (C). NC, negative control—untreated cells with media; PC, positive control—cells treated with 1% Triton-X-100. Data represent the mean ± SEM, n = 3, where * indicates statistical significance ($P < 0.05$).

exhibited some antifungal activity against all the fungal strains tested, with an MIC of 200 µg/ml. The *B frutescens* extracts showed antifungal activity against all the fungal strains with most activity found when tested against *C dubliniensis* with an MIC of 100 µg/ml.

Discussion

A ferox, *B frutescens*, and *A betulina* extracts have been used in traditional medicine to treat various ailments, including skin diseases. Anecdotal evidence for the traditional use of these extracts reports their application as traditional wound healing agents as well as for cosmetic and antibiotic use (Mabona and Van Vuuren, 2013; Pather et al., 2011). If these medicinal plant extracts are to be considered as antifungal agents, it is important that they are cytotoxic to the fungal strain while being selective to the host cells. The present study reports the concentrations of extracts with a proliferative or non-cytotoxic effect on normal skin cells while evaluating the concentrations of the extracts that were cytotoxic to fungi.

The results for cell proliferation showed time-dose responses, suggesting that the lower the dosage and the shorter the exposure time, the more effective the extract. Both *A ferox* and *B frutescens* demonstrated increased proliferation at lower concentrations (200 and 400 µg/ml) and decreased proliferation at higher concentrations (600 and 800 µg/ml). *Agathosma betulina* only exhibited a decrease in cell viability after longer exposure, with negligible changes observed in treatment concentrations after 24 hours. Similar studies have found that these extracts did not alter the cell viability of HaCaT cells after treatment (Leng et al., 2018; Pather and Kramer, 2012) rendering them non-cytotoxic on the skin cells. These results suggest that the optimum concentration for cell proliferation was between 200 and 400 µg/ml for *B frutescens* and 200 µg/ml for both *A ferox* and *A betulina* hydroethanolic extracts.

A compound is considered non-toxic when cell viability is higher than 80%. A weak cytotoxicity is associated with 80–60% cell viability, moderate cytotoxicity is linked to 60–40% viability, and a cell viability lower than 40% indicates cytotoxicity (López-García et al., 2014). To further validate that these extracts were not cytotoxic to the normal skin cells, an LDH cell cytotoxicity assay was performed. The results obtained from the assay confirmed that overall, the three selected ethanolic plant extracts displayed weak to non-cytotoxic properties. These extracts also had insignificant cytotoxic effects with low IC50 results for *A ferox* (IC50 599.89 µg/ml), *B frutescens* (IC50 768.21 µg/ml), and *A betulina* (IC50 771.06 µg/ml). Therefore, these extract dosages could potentially be considered safe to use on human skin. The results concur with previous studies, such as the one conducted by Fox et al. (2017), which showed that *A ferox* whole-leaf and gel materials caused no significant toxicity on HaCaT cells, rendering the plant safe to use on skin. Similar reports found that *A ferox* and *A betulina* extracts could also selectively inhibit microbial growth on the skin, with the extracts having no dermal toxicity or side effects (Mahomoodally, 2013; Van Vuuren, 2008). Bringmann et al. (2002) also reported that *B frutescens* constituents such as knipholore did not exhibit any cytotoxic effects on normal mammalian cells. The results suggest that these extracts could potentially be alternative agents that mitigate the inadvertent cytotoxicity of other antimicrobial and skin treatment agents to healthy skin cells (Ndlovu et al., 2021b).

To further elucidate the proliferative effects and non-cytotoxic effects of the extracts, it was important to investigate the potential morphological changes that occurred in the HaCaT cells upon exposure to the extracts. The H&E stain was performed to view the changes in cellular integrity and to monitor the potential induction of cell death (apoptosis) (Vorster et al., 2012). However, the direct mechanism of apoptosis was not fully explored in this study and can be viewed as a limitation. The H&E stain proved that all three extracts did not display any cytotoxic effects on the cells since the cellular structural integrity remained intact and unaffected after exposure. This suggests that the

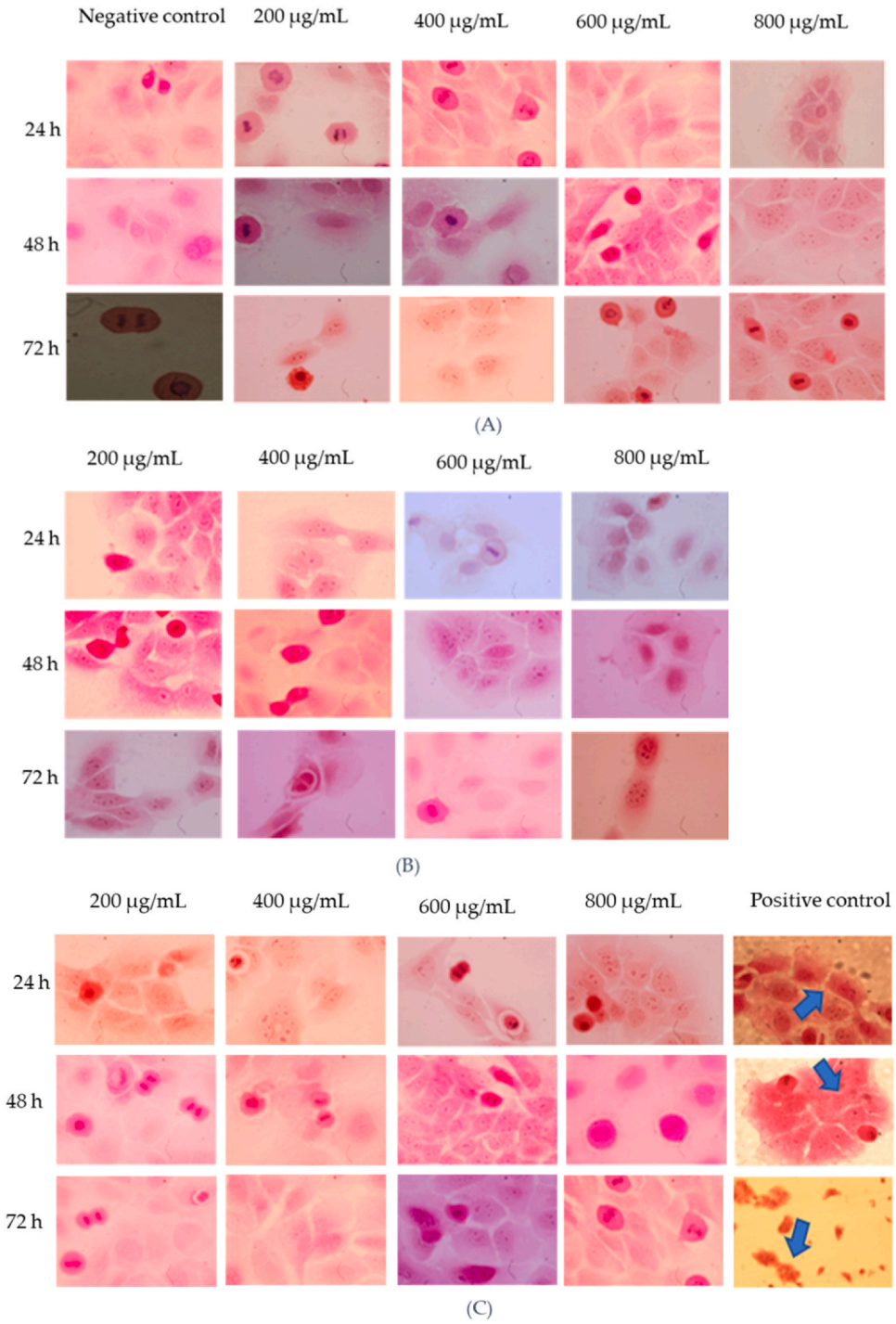

Fig. 3. Cellular morphology of the HaCaT cells after treatment with 200, 400, 600, and 800 µg/ml concentrations of *Aloe ferox* (A), *Bulbine frutescens* (B), and *Agathosma betulina* (C) hydroethanolic extracts. After treatment, the cells were stained with hematoxylin and eosin and photographed at 40 × magnification. Arrows show irregularly shaped cells.

Table 1
Antifungal activity of *Agathosma betulina*, *Aloe ferox*, and *Bulbine frutescens* hydroethanolic extracts.

Candida species	Plant extracts (µg/ml)			Fluconazole (µg/ml)
	A betulina	*A ferox*	*B frutescens*	
Candida albicans	50	200	200	200
Candida dubliniensis	100	200	100	200
Candida glabrata	200	200	200	200
Candida tropicalis	12.5	200	200	200

plant extracts are not harmful to the cells at any of the tested concentrations but could potentially cause cells to proliferate, a useful property if the extracts are to be used as antifungal agents (Agyare et al., 2016).

The three extracts exhibited antifungal activity against the fungal strains tested. The antifungal properties of the plant extracts were investigated, as it was imperative that the extracts were not toxic to host cells and selectively target fungi. According to the antimicrobial classification of Eloff (2021), an MIC < 0.02 mg/ml indicates remarkable activity, 0.021–0.04 mg/ml indicates excellent activity, 0.41–0.08 mg/ml indicates very good activity, 0.081–0.16 mg/ml indicates good activity, 0.161–0.32 mg/ml indicates average activity, and > 0.32 mg/ml indicates weak activity. Accordingly, the results of the present study found that *A betulina* displayed excellent activity against *C tropicalis* and very good activity against *C albicans*. *B frutescens* displayed good activity against *C dubliniensis,* while *A ferox* showed average activity against all four *Candida* species.

Very few studies have been conducted on the antifungal properties of these extracts against non-albicans *Candida* species. Some bioactive compounds isolated from medicinal plants play a vital role in modern medicine and are very potent drugs (2019). *B frutescens* was found to inhibit activity against the *Candida* strains, and this may be due to the presence of phytochemicals such as flavonoids, tannins, saponins and alkaloids in the plant. These are known to have antifungal activity and are toxic to most microbes (Chapagain et al., 2007; Rachuonyo et al., 2016). *A ferox* has the compound aloin that exhibits antimicrobial activity (MIC: 5 mg/ml) against *C albicans* (Kambiz and Afolayan, 2008). *A betulina* demonstrated potential antifungal activity with MIC values of 12 and 50 μg/ml against *C tropicalis* and *C albicans,* respectively. These results are similar to work conducted by Hübsch et al. (2014) on essential oils of *A betulina* that showed a strong antifungal activity (MIC: 2 mg/ml) against *C albicans*. *A betulina* has limonene and coumarin derivatives that reportedly have antimicrobial properties (Moolla et al., 2007). These three plant extracts could potentially be used as rich sources of antifungal compounds.

The three extracts were able to inhibit the growth of the different skin-related *Candida* species at concentrations of 200 μg/ml. The optimum concentration for skin cell proliferation was observed to be approximately 200 μg/ml for all three plant extracts. These results suggest that the extracts demonstrated antifungal activity against fungal skin infections while being non-toxic and maintaining the proliferation of healthy cells at the same concentration. However, further studies to determine the effects of these extracts on fungal-infected skin cells need to be explored, as this did not fall within the scope of this study. Overall, the results of the present study suggest that extracts from *A ferox*, *B frutescens*, and *A betulina* have the potential to be used as natural ingredients against cutaneous candidiasis.

Conclusions

The present study highlighted that the three hydroethanolic medicinal plant species investigated possess antifungal activity against *Candida* pathogens and have promising potential for use as antifungal agents. Effective antimicrobial activities were observed in the three extracts of the South African plants. The MIC results suggest that these plants are potentially safe for use as antifungals in traditional medicine; however, further *in vitro* and *in vivo* studies where skin cells are infected with the various *Candida* species should be conducted. The extracts demonstrated antifungal activity at concentrations that are favourable to the proliferation of epidermal cell growth, which is crucial to maintaining healthy skin. The present study investigated only the *in vitro* effect of the extracts on skin cells and fungal stains, and further studies examining the morphological changes would add an interesting perspective. Additionally, future studies addressing the active components and molecular mechanisms, as well as the determination of the minimum fungicidal concentration (MFC) of these extracts, need to be explored.

Ethical approval

Not applicable.

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CRediT authorship contribution statement

Abrantes Pedro: Writing – review & editing, Methodology. **Africa Charlene:** Writing – review & editing, Resources, Methodology. **Fisher (née Rahiman) Farzana:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Ndlovu Banele:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Klaasen Jeremy:** Writing – review & editing, Supervision, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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References

Agyare, C., Boakye, Y.D., Bekoe, E.O., Hensel, A., Dapaah, S.O., Appiah, T., 2016. African medicinal plants with wound healing properties. J. Ethnopharmacol. 177, 85–100.
Avula, B., Bae, J.-Y., Wang, Y.-H., Wang, M., Ali, Z., Khan, I.A., 2021. Chemical profiling and characterization of anthraquinones from two Bulbine species and dietary supplements using liquid chromatography–high resolution mass spectrometry. J. AOAC Int. 104 (5), 1394–1407.
Bizerra, F.C., Nakamura, C.V., De Poersch, C., Estivalet Svidzinski, T.I., Borsato Quesada, R.M., Goldenberg, S., Krieger, M.A., Yamada-Ogatta, S.F., 2008. Characteristics of biofilm formation by *Candida tropicalis* and antifungal resistance. FEMS Yeast Res. 8 (3), 442–450.
Bodede, O., Prinsloo, G., 2020. Ethnobotany, phytochemistry and pharmacological significance of the genus Bulbine (Asphodelaceae). J. Ethnopharmacol. 260, 112986.
Bringmann, G., Menche, D., Kraus, J., Mühlbacher, J., Peters, K., Peters, E.-M., Brun, R., Bezabih, M., Abegaz, B.M., 2002. Atropo-enantioselective total synthesis of knipholone and related antiplasmodial phenylanthraquinones. J. Org. Chem. 67 (16), 5595–5610.
Chang, S.H., Liu, J.Y., Hsiao, M.W., Yang, H.L., Wang, G.W., Ye, J.C., 2021. Protective effects of Ocimum gratissimumAqueous extracts on HaCaT cells against UVC-induced inhibition of cell viability and migration. Int. J. Med. Sci. 18 (9), 2086.
Chapagain, B.P., Wiesman, Z., Tsror, L., 2007. In vitro study of the antifungal activity of saponin-rich extracts against prevalent phytopathogenic fungi. Ind. Crops Prod. 26 (2), 109–115.
Chen, W., Van Wyk, B.-E., Vermaak, I., Viljoen, A.M., 2012. Cape aloes—a review of the phytochemistry, pharmacology and commercialisation of Aloe ferox. Phytochem. Lett. 5 (1), 1–12.
Coopoosamy, R.M., 2011. Traditional information and antibacterial activity of four Bulbine species (Wolf). Afr. J. Biotechnol. 10 (2), 220–224.
Declara, S.M., Agnetti, F., Roccabianca, P., Squassino, C., Porporato, F., Zanna, G., 2022. Mucocutaneous and cutaneous generalized candidiasis in a thymectomized dog. Med. Mycol. Case Rep. 35, 39–42.
Eloff, J.N., 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 64 (8), 711–713.
Eloff, J.N., 2021. A proposal towards a rational classification of the antimicrobial activity of acetone tree leaf extracts in a search for new antimicrobials. Planta Med. 87 (10/11), 836–840.
Espinosa-Hernández, V.M., Morales-Pineda, V., Martínez-Herrera, E., 2020. Skin infections caused by emerging Candida species. Curr. Fungal Infect. Rep. 14, 99–105.
Fajinmi, O., Kulkarni, M., Benická, S., Zeljković, S.Ć., Doležal, K., Tarkowski, P., Finnie, J., Van Staden, J., 2019. Antifungal activity of the volatiles of *Agathosma betulina* and *Coleonema album* commercial essential oil and their effect on the morphology of fungal strains *Trichophyton rubrum* and *T. mentagrophytes*. S. Afr. J. Bot. 122, 492–497.
Fox, L.T., Mazumder, A., Dwivedi, A., Gerber, M., Du Plessis, J., Hamman, J.H., 2017. In vitro wound healing and cytotoxic activity of the gel and whole-leaf materials from selected aloe species. J. Ethnopharmacol. 200, 1–7.

Frum, Y., Viljoen, A., 2006. In vitro 5-lipoxygenase and anti-oxidant activities of South African medicinal plants commonly used topically for skin diseases. Skin Pharmacol. Physiol. 19 (6), 329–335.
Gediya, S.K., Mistry, R.B., Patel, U.K., Blessy, M., Jain, H.N., 2011. Herbal plants: used as a cosmetics. J. Nat. Prod. Plant Resour. 1 (1), 24–32.
Glen, H., Hardy, D., 2000. Aloe, Aloeaceae (first part). Flora S. Afr. 5 (1.1).
Gnat, S., Łagowski, D., Nowakiewicz, A., 2021. Genetic predisposition and its heredity in the context of increased prevalence of dermatophytoses. Mycopathologia 186, 163–176.
Hübsch, Z., Van Zyl, R., Cock, I., Van Vuuren, S., 2014. Interactive antimicrobial and toxicity profiles of conventional antimicrobials with Southern African medicinal plants. S. Afr. J. Bot. 93, 185–197.
Jin, L., Cao, Z., Wang, Q., Wang, Y., Wang, X., Chen, H., Wang, H., 2018. MDR1 over-expression combined with ERG11 mutations induce high-level fluconazole resistance in *Candida tropicalis* clinical isolates. BMC Infect. Dis. 18, 1–6.
Kambiz, L., Afolayan, A., 2008. Extracts from Aloe ferox and Withania somnifera inhibit *Candida albicans* and *Neisseria gonorrhoea*. Afr. J. Biotechnol. 7 (1), 012–015.
Kühbacher, A., Burger-Kentischer, A., Rupp, S., 2017. Interaction of Candida species with the skin. Microorganisms 5 (2), 32.
Kumar, P., Nagarajan, A., Uchil, P.D., 2018. Analysis of cell viability by the lactate dehydrogenase assay. Cold Spring Harb. Protoc. 2018 (6), 465–468.
Lall, N., Kishore, N., 2014. Are plants used for skin care in South Africa fully explored? J. Ethnopharmacol. 153 (1), 61–84.
Leng, H., Pu, L., Xu, L., Shi, X., Ji, J., Chen, K., 2018. Effects of aloe polysaccharide, a polysaccharide extracted from Aloe vera, on TNF-α-induced HaCaT cell proliferation and the underlying mechanism in psoriasis. Mol. Med. Rep. 18 (3), 3537–3543.
Lis-Balchin, M., Hart, S., Simpson, E., 2001. Buchu (*Agathosma betulina* and A. crenulata, rutaceae) essential oils: their pharmacological action on guinea-pig ileum and antimicrobial activity on microorganisms. J. Pharm. Pharmacol. 53 (4), 579–582.
López-García, J., Lehocký, M., Humpolíček, P., Sáha, P., 2014. HaCaT keratinocytes response on antimicrobial atelocollagen substrates: extent of cytotoxicity, cell viability and proliferation. J. Funct. Biomater. 5 (2), 43–57.
Lu, H., Shrivastava, M., Whiteway, M., Jiang, Y., 2021. *Candida albicans* targets that potentially synergize with fluconazole. Crit. Rev. Microbiol. 47 (3), 323–337.
Mabona, U., Van Vuuren, S., 2013. Southern African medicinal plants used to treat skin diseases. S. Afr. J. Bot. 87, 175–193.
Macià, M.D., Del Pozo, J.L., Díez-Aguilar, M., Guinea, J., 2018. Microbiological diagnosis of biofilm-related infections. Enferm. Infecc. Y. Microbiol. Clin. (Engl. Ed.) 36 (6), 375–381.
Mahomoodally, M.F., 2013. Traditional medicines in Africa: an appraisal of ten potent African medicinal plants. Evid. Based Complement. Altern. Med. 2013 (1), 617459.
Masondo, N., Makunga, N., 2019. Advancement of analytical techniques in some South African commercialized medicinal plants: current and future perspectives. S. Afr. J. Bot. 126, 40–57.
Moolla, A., Van Vuuren, S., Van Zyl, R., Viljoen, A.M., 2007. Biological activity and toxicity profile of 17 Agathosma (Rutaceae) species. S. Afr. J. Bot. 73 (4), 588–592.
Nascente, Pd.S., Meinerz, A.R.M., Faria, R.Od, Schuch, L.F.D., Meireles, M.C.A., Mello, J.R.Bd, 2009. CLSI broth microdilution method for testing susceptibility of Malasseziapachydermatis to thiabendazole. Braz. J. Microbiol. 40, 222–226.
Ndlovu, B., Africa, C., Klaasen, J., Rahiman, F., 2021a. Does South Africa hold the key to the development of alternative treatments for resistant dermatophyte infections? A review. J. Herb. Med. 29, 100474.
Ndlovu, B., De Kock, M., Klaasen, J., Rahiman, F., 2021b. In vitro comparison of the anti-proliferative effects of Galenia africana on human skin cell lines. Sci. Pharm. 89 (1), 12.
Ng'uni, T.L., dos Santos Abrantes, P.M., McArthur, C., Klaasen, J.A., Fielding, B.C., 2022. Evaluation of synergistic anticandidal activity of *Galenia africana* extract and fluconazole against *Candida albicans* and *Candida glabrata*. J. Herb. Med. 32, 100503.
Pather, N., Kramer, B., 2012. *Bulbine natalensis* and *Bulbine frutescens* promote cutaneous wound healing. J. Ethnopharmacol. 144 (3), 523–532.
Pather, N., Viljoen, A.M., Kramer, B., 2011. A biochemical comparison of the in vivo effects of *Bulbine frutescens* and *Bulbine natalensis* on cutaneous wound healing. J. Ethnopharmacol. 133 (2), 364–370.
Penduka, D., Okoh, O.O., Okoh, A.I., 2011. In-vitro antagonistic characteristics of crude aqueous and methanolic extracts of *Garcinia kola* (Heckel) seeds against some Vibrio bacteria. Molecules 16 (4), 2754–2765.
Pereira, Fd.O., 2021. A review of recent research on antifungal agents against dermatophyte biofilms. Med. Mycol. 59 (4), 313–326.
Rachuonyo, H., Kamau, D., Ogola, P., Arika, W., Wambani, J., Nyamache, A., 2016. In vitro Antifungal activity of leaf extracts from *Aloe secundiflora*, *Bulbine frutescens*, *Vernonia lasiopus* and *Tagetes minuta* against *Candida albicans*. Med. Aromat. Plants 5 (2), 2167.
Ribeiro, A.S., Estanqueiro, M., Oliveira, M.B., Sousa Lobo, J.M., 2015. Main benefits and applicability of plant extracts in skin care products. Cosmetics 2 (2), 48–65.
Saotome, K., Morita, H., Umeda, M., 1989. Cytotoxicity test with simplified crystal violet staining method using microtitre plates and its application to injection drugs. Toxicol. In Vitro 3 (4), 317–321.
Sharma, L., Agarwal, G., Kumar, A., 2003. Medicinal plants for skin and hair care. Indian J. Tradit. Knowl. 2 (1), 62–68.
Silva, S., Negri, M., Henriques, M., Oliveira, R., Williams, D.W., Azeredo, J., 2012. *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis*: biology, epidemiology, pathogenicity and antifungal resistance. FEMS Microbiol. Rev. 36 (2), 288–305.
Van Vuuren, S., 2008. Antimicrobial activity of South African medicinal plants. J. Ethnopharmacol. 119 (3), 462–472.
Van Wyk, B.-E., 2008. A broad review of commercially important southern African medicinal plants. J. Ethnopharmacol. 119 (3), 342–355.
Viljoen, A.M., Van Vuuren, S.F., Gwebu, T., Demirci, B., Başer, K.H.C., 2006. The geographical variation and antimicrobial activity of African wormwood (*Artemisia afra* Jacq.) essential oil. J. Essent. Oil Res. 18, 19–25.
Vorster, C., Stander, A., Joubert, A., 2012. Differential signaling involved in *Sutherlandia frutescens*-induced cell death in MCF-7 and MCF-12A cells. J. Ethnopharmacol. 140 (1), 123–130.
Wilkinson, J., Brown, A., 1999. Horse Chestnut–*Aesculus hippocastanum*: potential applications in cosmetic skin-care products. Int. J. Cosmet. Sci. 21 (6), 437–447.
Wong, S.S., Samaranayake, L.P., Seneviratne, C.J., 2014. In pursuit of the ideal antifungal agent for Candida infections: high-throughput screening of small molecules. Drug Discov. Today 19 (11), 1721–1730.
Zapata, P.J., Navarro, D., Guillén, F., Castillo, S., Martínez-Romero, D., Valero, D., Serrano, M., 2013. Characterisation of gels from different Aloe spp. as antifungal treatment: Potential crops for industrial applications. Ind. Crops Prod. 42, 223–230.
Zhao, Y., Sun, Y.N., Lee, M.J., Kim, Y.H., Lee, W., Kim, K.H., Kim, K.T., Kang, J.S., 2016. Identification and discrimination of three common Aloe species by high performance liquid chromatography–tandem mass spectrometry coupled with multivariate analysis. J. Chromatogr. B 1031, 163–171.